Magnetic Beads-TUBE2 (High Capacity)

Cat. # UM502M

Background:

Based on protein domains known to possess an affinity for ubiquitin, Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitylated proteins. TUBEs display up to a 1000-fold increase in affinity for poly-ubiquitin moieties over the single ubiquitin binding associated domain (UBA). In addition, TUBEs display a protective effect on polyubiquitylated proteins, allowing for detection at relatively low abundance. These properties effectively "capture" protein in its polyubiquitin state. UM502M was designed by coating high-capacity magnetic beads to allow superior enrichment of poly-ubiquitylated proteins along with minimizing non-specific binding to proteins in tissue and cellular lysates.

Applications:

- Pull down of poly-ubiquitylated proteins from cell lines, tissues, and organs
- Protection of poly-ubiquitylated proteins from both deubiquitylation and degradation by the proteasome
- Bead-based ELISA to study ubiquitinated proteome
- Label free mass spectrometry ubiquitinated proteomic analysis

Product Information

Affinity tag: N/A

Purity: (prior to coupling) > 95% by SDS-AGE

Molecular Weight: N/A

Quantity: 1 mL magnetic beads

Physical State: Liquid

Storage: +4 C. Avoid storage at lower temperatures

References

1. Hjerpe, R, Aillet, F, Lopitz-Otsoa, F, Lang, V, England, P, and Rodriguez, MS., Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. EMBO Rep. 10,1250-1258 (2009).
2. Stormo, Adrienne ED, Farbod Shavarebi, Molly FitzGibbon, Elizabeth M. Earley, Hannah Ahrendt, Lotus S. Lum, Erik Verschueren et al (2022) "The E3 ligase TRIM1 ubiquitinates LRRK2 and controls its localization, degradation, and toxicity." Journal of Cell Biology 221, no. 4.
3. Sarbanes, Stephanie L., Vincent A. Blomen, Eric Lam, Søren Heissel, Joseph M. Luna, Thijn R. Brummelkamp, Erik Falck-Pedersen, H-Heinrich Hoffmann, and Charles M. Rice. (2021) "E3 ubiquitin ligase Mindbomb 1 facilitates nuclear delivery of adenovirus genomes." Proceedings of the National Academy of Sciences 118, no. 1: e2015794118

Representative Data for UM502M – High capacity TUBE2 magnetic beads

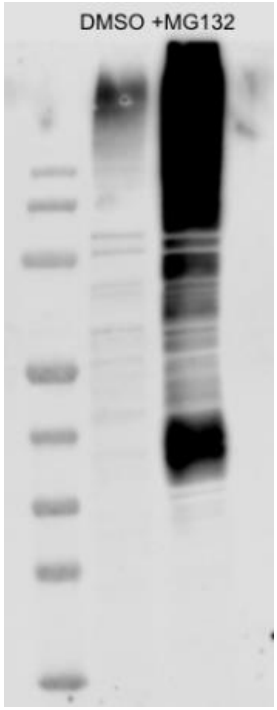

Figure 1. Enrichment of total ubiquitinated proteome using UM502M. 100 µL of UM502M beads were added to 300 µg of cell lysates derived from HeLa cells treated with: DMSO and 1 µM MG-132 treated for 4 hours. The data represents overnight enrichment of both DMSO and MG-132 treated lysates using UM502M at 4°C on a end-to-end rotator. Characteristic enhanced ubiquitination smears were observed for proteasome inhibitor (MG-132) treated samples suggest robust pulldown of total poly-ubiquitinated proteome. The enriched beads were resuspended in 30 µL of 1X Laemmli sample buffer prior to loading on a 10% SDS-PAGE and western blotting using anti-ubiquitin (VU101).